Anti-Ulcer Activity of the Leaf Extracts of *Borreria ocymoides* in Rats

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Abstract

The anti-ulcer properties of the leaf extracts of Borreria ocymoides Burm. F. (Rubiaceae) was investigated. Sixty (60) albino rats of either sex weighing 160-216g and fifteen (15) albino mice weighing 25-36 g were used for the study. Standard pharmacological methods were used to carry out acute toxicity (LD_{50}) and phytochemical screening of the plant extract. Gastrointestinal and anti-ulcer studies were performed using standard methods. The extract had an oral LD_{50} of > 8000mg/kg in mice. Phytochemical screening revealed the presence of abundant amounts of carbohydrates, reducing sugars, alkaloids, tannins, proteins, and moderate amounts of flavonoids, terpenoids, saponins and glycosides. There were also trace amounts of resins and steroids. The aqueous and methanolic extracts of Borreria ocymoides (AEBO and MEBO) at dose level of 800mg/kg orally, increased gastro-intestinal motility by 6.1% and 22.5% respectively. This increase was not significant when compared with the negative control (p > 0.05). The laxative drug, bisacodyl (5mg/kg), increased motility by 17.4%. However, when bisacodyl (5mg/kg) was administered concomitantly either with AEBO (800mg/kg) or MEBO (800mg/kg), gastrointestinal motility was increased significantly by 67.8% or 70.1% respectively (p < 0.001). Furthermore, the AEBO and MEBO (800mg/kg b.w, p.o) produced significant ulcer protective potency against indomethacin and histamine induced gastric ulceration when compared with the negative control (p < 0.001). The MEBO had comparable ulcer protective potency with cimetidine. Both extracts possess gastric ulcer protective potency and justifies the use of this plant for ulcer treatment.

Keywords: *Borreria ocymoides*, Anti-ulcer, Indomethacin, Histamine, Ulcer index, Gastric motility

Introduction

Nature has been the source of medicinal agents since the beginning of man. In Nigeria, traditional medicine is currently well acknowledged and established as a viable profession (Kafaru, 1994). Gastric ulcer is one of the most prevalent gastrointestinal disorders (Valle, 2005). The pathophysiology of gastric ulcer involves an imbalance between aggressive (acid, pepsin, and *H. pylori*) and protective or defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) (Hoogerwerf and Pasricha, 2001). An estimated 15,000 deaths occur each year as a result of peptic ulcer disease (Valle, 2005). The goal of gastric ulcer treatment is to reduce the production of gastric acid and to stimulate gastric mucosal protection (Valle, 2005).

Currently, there is astounding progress in the knowledge of the pathophysiology of gastric ulcers and this has made it possible to research and develop better drug therapy. This has resulted in the development of proton pump inhibitors, histamine (H_2) receptor antagonists, and drugs affecting mucosal barrier and prostaglandin analogues (Dharman and Palit, 2006). Unfortunately, clinical evaluation of these drugs showed development of tolerance and incidence of relapses and side effects that make their efficacy arguable. This has led to the search for novel molecules present in herbs that offer better protection and decreased incidence of relapse. A folk medicine possessing fewer side effects is a desirable for better and safer approach for the management of gastric ulcer disease. Moreover, pharmacoeconomic considerations have not been favourable in the prospects of procuring the allopathic drugs by patients in developing countries. Consequently, a large number of herbs and spices have been evaluated by various researchers for their anti-ulcer effects with favourable outcome (Al-Yahya *et al.,* 1990; Alvarez *et al.,* 1999; Anoop and Jegadeesan, 2003; Sairam *et al.,* 2003; Al-Mofleh et al., 2005; Dharmani *et al.,* 2005; Okwuosa *et al.,* 2006; Al-Mofleh *et al.,* 2006; Al- Mofleh *et al.,* 2008).

Borreria ocymoides Burm. F. is a plant commonly referred to as Lindrina and belongs to the family- Rubiaceae. The description of the plant morphology has been documented (Hutchinson and Dalziel, 1954). The extract of the leaves has remarkable inhibitory activity against drug resistant *Plasmodium falciparum* (Kafaru, 1994). The stems and leaves are used to treat various inflammatory diseases such as asthma, rheumatoid arthritis, and swelling associated with sickle cell anaemia in children (Ohiri, 2004). It is used to treat jaundice and the aqueous leaf extract is used in folklore as haematinic, anti-ulcer, and anti-fungal and there have been claims of considerable success with the use of this plant. Consequently, this could have great potential for the finding of a novel anti-ulcer ingredient. Therefore, the present study was undertaken to determine the gastroprotective activity of the aqueous and methanol extracts of B. ocymoides against indomethacin and histamine induced gastric ulceration in rats.

Materials and Methods

Drugs and chemicals: Histamine, Atropine, (Sigma Chemical Company, USA), Cimetidine (Samoab Pharmaceuticals Ltd), Absolute Methanol (Fischer Scientific, Ltd), Bisacodyl (Medlax Pharmaceuticals, Mumbai, India), Indomethacin (Dumax Pharmaceuticals)

Animals: Sixty (60) albino wistar rats (160-216g) and fifteen (15) albino mice were obtained from the Animal House of the College of Medicine, University of Nigeria, Enugu Campus. These animals were kept in clean, gauzed cages and acclimatized for two weeks at the Animal House of the College of Medicine, University of Nigeria, Enugu Campus, under standard condition of temperature (25±3° C) and a 12:12 hour light/dark periodicity. The animals were allowed free access to standard pellets (Guinea feed®) and clean water *ad libitum.* All the animals were handled in this study according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings (APS, 2002).

Plant collection: The plant leaves were collected from a field in Enugu campus of the University of Nigeria in the month of May, 2008 and identified (Oliver, 1960). A voucher specimen was deposited at the Herbarium, Department of Botany, University of Nigeria, Nsukka (UNH /578) for future reference. The leaves were air-dried under a shade and powdered using an electric blender/mill grater III (model MS-223, Taiwan).

Aqueous extraction: 500g of dried fresh leaves were macerated using a mechanical grinder. 250mls of clean water was added to the macerated leaves and homogenized using a wooden rod as stirrer. The homogenate was strained through muslin. The filtrate was further concentrated by allowing it to stand overnight in an oven at 30°C and stored at 4°±2°C until required. The aqueous extract gave a percentage yield of 11.4%.

Methanol extraction: 800g of powdered leaves of B. ocymoides was macerated in 2 litres of 80% methanol for 48hrs. The extract was filtered through a Whatman no. 1 filter paper. The filtrate was then evaporated to dryness on a rotary evaporator (Model type 349/2 Corning Ltd). The dried residue was stored at 4°±2°C. A yield of 15.5 % of the methanol extract was obtained. A known quantity of the ME was dissolved in 3% aqueous suspension of Tween 80 to obtain the desired concentration for the study.

Acute toxicity (LD_{50}) testing: The acute toxicity testing was performed according to the procedure of Lorke (1983)

Phytochemical screening: Phytochemical screening tests were done using standard methods (Trease and Evans, 1989).

Indomethacin induction of ulcer: The model of ulcer induction adopted for the screening was the indomethacin model. Prior to the test, the rats were starved of food for 24 hours but had free access to water. Sixteen (16) animals were employed and grouped into four groups (A – D) of 4 rats each. Group A received 10ml/kg of 3% tween 80 orally (p.o) and served as negative control. Group B received cimetidine (100mg/kg p.o), group C received 800mg/kg body weight of the aqueous extract, while group D received 800mg/kg of the methanol extract (p.o). Thirty minutes later, ulcers were induced by the oral administration of indomethacin (40mg/kg), suspended in 3% tween 80, to the different groups of animals. After 8 hours, the animals were euthanized and their stomach opened along the greater curvature. The stomach of each animal was rinsed under a stream of water and pinned flat on a cork board. The ulcers were viewed with the aid of a magnifying lens(x 10) and each given a severity rating (Main and Whittle, 1975) as follows: <1mm = 1(pin point), >1mm<2mm = 2 and >2mm<3mm = 3.

The overall total divided by a factor of 10 was designated as the ulcer index (UI) for that stomach. The percentage ulcer protection was calculated as follows (Suzuki et al., 1976):

Percentage ulcer protection = 1- ulcer index for test agent X 100 / ulcer index for negative control.

Histamine induced ulceration: Ulceration was induced in the experimental animals with histamine (0.5mg/kg) administered intraperitoneally (i.p) to the rats. Sixteen (16) albino rats of either sex were employed for the study. The animals were divided into four (4) groups (A-D) of four (4) rats per group. Group A received 3% Tween 80 (5ml/kg) and served as negative control. Group B received Cimetidine (100mg/kg) and served as positive control. Group C received AEBO (800mg/kg) while group D was given 800mg MEBO per kg body weight. All the drugs were administered orally. Thirty (30) minutes later, ulcer was induced by the administration of histamine (0.5mg/kg i.p) to all the animals. After eight (8) hours, the animals were euthanized; their stomach excised, and cut open along the greater curvature, rinsed with water, and pinned on a cork board for observation. The ulcers were viewed with the aid of a magnifying lens(x 10) and each given a severity rating (Main and Whittle, 1975).

Gastrointestinal motility test: Twenty-eight (28) albino rats of either sex were randomly assigned to seven (7) groups (A-G) of four animals each. The animals were starved for 24 hours prior to the experiment but had free access to clean water. Group A received 3% aqueous suspension of tween 80 (20ml/kg), group B received atropine (10mg/kg) and served as positive control group, group C received AEBO (800mg/kg), while group D received MEBO (800mg/kg) Group E received bisacodyl (5mg/kg) while group F and G received bisacodyl (5mg/kg) plus 800mg/kg AEBO and 800mg/kg MEBO respectively. All administrations were by the oral route. Five minutes after drug administration, 0.5ml of a 10% activated charcoal in 3% aqueous

suspension of tween 80 was administered to each animal orally. The animals were sacrificed 30 minutes later and their abdomen opened. The percentage distance of the intestine (from the pylorus to the caecum) traveled by the charcoal plug in the treatment groups were determined as described by Akah *et al*. (1997).

Statistical analysis: The results were expressed where appropriate as Mean ± Standard error of mean. The mean values of test groups were compared with those of the control group and considered significant at $p < 0.05$; $p < 0.01$; $p < 0.001$ using the student's t-test.

Result

Acute toxicity screening (LD_{50}) showed that the extract had an oral LD_{50} > 8000mg/kg in mice. Phytochemical screening revealed the presence of abundant amounts of carbohydrates, reducing sugars, alkaloids, tannins, proteins, and moderate amounts of flavonoids, terpenoids, saponins and glycosides. There were also trace amounts of resins and steroids (Table 1).

Table 1: Phytochemical profile of the extract

Phytochemicals	Scores
Carbohydrates	+++
Reducing sugars	+++
Alkaloids	+++
Glycosides	++
Saponins	++
Tannins	+++
Flavonoids	++
Resins	+
Proteins	+++
Oil	-
Steroids	+
Terpenoids	++
Acidic compounds	-

Key: *- = absent; + = Low concentration; ++ = Moderate concentration; +++ = High concentration*

When indomethacin (40mg/kg) was given orally to the animals, the rats developed ulcers at the end of eight (8) hours and the ulcer indices are shown in Table 2. Both AEBO and MEBO offered significant ulcer protective potencies against indomethacin induced ulceration with mean ulcer indices of 7.75 ± 1.89 and 3.00 ± 0.41 respectively when compared with the ulcer index of the negative control (70.75 ± 3.94; $p < 0.001$). The MEBO had comparable ulcer protective potency with the positive control (Cimetidine). The AEBO and MEBO also exhibited significant ulcer protective activities against histamine induced ulceration with mean ulcer indices of 5.75 ± 1.11 and 4.00 ± 0.71 respectively when compared with the ulcer index of the negative control (20.75 ± 3.33; $p < 0.01$ (Table 3).

Table 3 also indicated that the aqueous extract had a significantly lower anti-ulcer activity when compared with that of the positive control (UI = 1.00 ± 0.41; $p < 0.05$) while the MEBO had comparable potency with the positive control drug. The result of gastrointestinal motility studies (Table 2) showed that both AEBO and MEBO increased GIT motility by 6.1 and 22.5 % respectively. However, this increase was not significant when compared with the negative control ($p > 0.05$). This increase in motility was enhanced by concomitant administration of the extracts with bisacodyl ($p < 0.001$).

Discussion

The extracts of *Borreria ocymoides* are used in folk medicine for the treatment of ulcers, fungal infections, malaria, anaemia, sickle cell disease, inflammation, and jaundice. The high margin of safety was indicated by the fact that in the LD_{50} determination, no death occurred even after an oral dose of 8000mg/kg in rats. The aqueous and methanol extracts showed potency as anti-ulcer agents against indomethacin and histamine induced ulceration in rats. This anti-ulcer potency of the plant could be as a result of its phytochemical constituents. Hydrolysable tannins contain glucose moiety and have been used internally as astringent and as heavy metal antidote (Merck Index, 1989). Tannins being astringent may precipitate microproteins on the site of ulcer thereby forming an impervious protective pellicle on the lining to resist the attack of proteolytic enzymes (John and Onabanjo, 1990). This could be likened to the effect of drugs such as sucralfates which act by providing a cytoprotective defense against acid peptic digestion. This is consistent with a previous report that partly attributed the anti-ulcer activity of crude rhizome extract of *Microgramma squamulosa* to the astringent action of tannins (Suffredini *et al.,* 1999). Anti-oxidants play a protective role against cellular damage by scavenging free radicals (Szabo, 1989). Reduction in the concentration of free radicals enhances cyclooxygenase activity thereby increasing prostaglandin synthesis (Halliwel and Gutteridge, 1987). Ulcer induction by indomethacin results from inhibition of synthesis of prostaglandins which leads to overproduction of leucotrienes and other products of the 5-lipoxygenase pathway. These agents break mucosal barrier, stimulate an increase in gastric mucosal permeability to H^+ and Na^+ ions, consequently reducing the transmucosal potential difference and induce formation of erosions and ulcers (AL-Mofleh *et al*., 2008). However, there are multiple aetiologic factors in ulcer pathogenesis and the ability of the extracts to protect against indomethacin and histamine induced ulceration may indicate their ability to inhibit one or more multiple inciting stimuli in ulcerogenesis. Flavonoids have been shown to possess anti-ulcerogenic and anti-ulcer activities (Tamotsu et al., 1978).

Furthermore, alkaloids were abundant in the extract and alkaloids are substances known to affect the integrity of the mucous membrane (Oliver, 1960). Alkaloids such as hyoscine-N-methyl bromide have been shown to suppress acid secretion (BNF, 1996). It is likely that the protective activity of the extract against histamine induced ulceration is as a result of suppression of acid secretion. Anthraquinones, steroids, and terpenoids are also present in *B. ocymoides* and these substances have been shown to possess anti-ulcer activities (Gaginella, 1992).

Table 2: Ulcer Indices (UI) of treatment groups compared with negative control in indomethacin induced ulceration

Treatment group	Dose mg/kg	Number of rats	Number that developed ulcer	Ulcer Index (mean±SEM)	% Ulcer protection
3% Tween 80	5ml/kg	4	4	70.75 ± 3.94	Nil
Cimetidine	100	4	4	2.25 ± 1.11***	96.9
Aqueous extract	800	4	4	7.75 ± 1.89***	89
Methanolic extract	800	4	4	3.00 ± 0.41***	95.8

*** $p < 0.001$

Table 3: Ulcer Indices (UI) of treatment groups compared with negative control in histamine induced ulceration

Treatment group	Dose mg/kg	Number of rats	Number that developed ulcer	Ulcer Index (mean±SEM)	% Ulcer protection
3% Tween 80	5ml/kg	4	4	20.75 ± 3.33	Nil
Histamine	100	4	4	1.00 ± 0.41***	95.2
Aqueous extract	800	4	4	5.75 ± 1.11**	72.3
Methanolic extract	800	4	4	4.00 ± 0.71**	80.7

**$p < 0.01$; ***$p < 0.001$

Table 4: Comparison of the percentage distance traveled by the charcoal plug in treatment groups with negative control in the gastrointestinal motility studies

Treatment group	Dose (mg/kg)	% Distance traveled (Mean ± SEM)	% Increase in GIT motility
3% Tween 80	10ml/kg	58.76 ± 5.36	Nil
Atropine	10	13.43 ± 4.06**	- 77.2
AEBO	800	62.33 ± 8.87	6.1
MEBO	800	72.00 ± 6.54	22.5
Bisacodyl	5	68.98 ± 2.60	17.4
Bisacodyl and AEBO	5/800	98.63 ± 1.38***	67.8
Bisacodyl and MEBO	5/800	100.00 ± 0.00***	70.1

**$p < 0.01$; ***$p < 0.001$

The extracts produced modest increase in gastrointestinal motility. However, this increase became profound when these extracts were given concomitantly with bisacodyl suggesting synergy. A drug could have a purgative or laxative effect and this depends on the dose. Laxative properties of so many medicinal plants have been linked to their anthraquinone and emodin content (Muller-Lissner, 1993).

The results of the present study establish the anti-gastric ulcer properties of the aqueous and methanol extracts of Borreria ocymoides leaves and consequently substantiates the use of this plant in stomach disorders in Nigerian traditional pharmacopoeia.

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